

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. – 12. Cancelled.

13. (Previously Presented) A method for producing an evolved protein comprising:

- a) ~~generating a directed genetic modification in a gene of interest in an initial microorganism preparing to yield a modified microorganism by genetic modification of cells of an initial microorganism,~~ wherein the production or consumption of a ~~metabolite~~ substrate is inhibited when the ~~initial~~ modified microorganism is grown on a defined medium, wherein the ability of the modified microorganism to grow is impaired;
- b) culturing the modified microorganism obtained in step (a) on the defined medium ~~causing~~ allowing the modified microorganism to evolve a compensatory metabolic pathway[[s,]] to compensate for the impaired growth, ~~where~~ wherein the defined medium can contain a co-substrate promoting the evolution; and
- c) selecting ~~the~~ an evolved microorganism ~~of~~ from step (b) able to grow on the defined medium, wherein at least one protein has evolved in the metabolic pathway compensating for the inhibition the evolved allowing the modified microorganism produces an evolved protein to proliferate
- d) isolating the evolved protein.

14. (Original) The method as claimed in Claim 13, characterized in that the produced evolved protein is purified.

15. – 37 Cancelled.

38. (New) The method of claim 13, wherein the gene of interest is a homologous gene or a heterologous gene.

39. (New) The method of claim 13, wherein the evolved protein is a homologous protein or a heterologous protein.

40. (New) The method of claim 13, wherein the defined medium is substantially free of the substrate the production or consumption of which is inhibited in the modified microorganism.
41. (New) The method of claim 13, wherein, in step (b) a co-substrate is added to the defined medium.
42. (New) The method of claim 13, wherein, in step (c) a co-substrate is added to the defined medium.
43. (New) The method of claim 13, wherein the protein having evolved in the compensatory pathway is encoded by a gene being a homologous gene or a heterologous gene.
44. (New) The method of claim 13, wherein the genetic modification comprises the directed mutation or deletion of a gene of interest or the directed modification of a promoter in the gene of interest.
45. (New) The method of claim 13, wherein the genetic modification consists in the removal of most of the gene of interest.
46. (New) The method of claim 13, wherein the gene of interest is replaced with a selection marker gene.
47. (New) The method of claim 13, wherein the microorganism is selected among bacteria, yeasts and fungi.
48. (New) The method of claim 13, wherein the microorganism is *Aspergillus sp.*, *Bacillus sp.*, *Brevibacterium sp.*, *Clostridium sp.*, *Corynebacterium sp.*, *Escherichia sp.*, *Gluconobacter sp.*, *Pseudomonas sp.*, *Rhodococcus sp.*, *Saccharomyces sp.*, *Streptomyces sp.*, *Xanthomonas sp.*, or *Candida sp.*
49. (New) The method of claim 13, wherein the microorganism is *E. coli* and *C. glutamicum*.